

**WHAT IS CLAIMED IS:**

1. A method for testing to determine the presence or amount of an analyte in a biological sample collected from a subject, comprising the steps of:
  - simultaneous on-line extraction of the analyte from the sample into a supercritical fluid by,
  - collecting the sample from the subject and placing the sample into an extraction chamber;
  - exposing the sample in the extraction chamber to a supercritical fluid to extract the analyte from the sample;
  - eluting the extracted analyte from the extraction chamber;
  - collecting the eluate containing the extracted analyte;
  - testing the eluate to determine the presence or amount of the analyte; and,
  - determining that if the analyte is present said analyte was present in the sample.
2. A supercritical fluid extraction method for screening a subject to determine the possible presence of a drug analyte, wherein the drug usage by said subject is unknown, comprising the steps of collecting a biological sample from the subject; simultaneously extracting any analyte present in the sample by first treating the sample with a derivatizing agent effective to neutralize the chemical charge of one or more substituent charged groups in said analyte and thereafter extracting any derivatized drug analytes in said sample into a supercritical fluid; and, collecting said supercritical fluid and testing to detect the presence or amount of said derivatized analyte.
3. The method of claim 1 or 2, wherein the supercritical fluid is selected from group consisting of carbon dioxide, nitrous oxide, pentane and butane.
4. The method of claim 2, wherein said analyte substituent groups are selected from the group consisting of hydroxyl, carboxyl, amide, amine and thiol.

5. The method of claim 2, wherein said derivatizing agent is selected from the group consisting of an alkylating reagent, an acylating reagent and a silylating reagent.
6. The method of claim 3, wherein said supercritical fluid comprises CO<sub>2</sub>.
7. The method of claim 6, wherein said supercritical fluid additionally comprises CO<sub>2</sub> about 1% (v/v) to about 30% (v/v) of a modifier solution.
8. The method of claim 7, wherein said modifier comprises an organic solvent selected from the group consisting of methanol, dichloromethane, ethanol, ethyl acetate, isopropanol, and hexane.
9. The method of claim 7, wherein said CO<sub>2</sub> supercritical fluid additionally comprises about 0.5% (v/v) to about 1.2% (v/v) of an additive solution and/or water.
10. The method of claim 9, wherein said additive solution comprises an amine additive selected from the group consisting of methylamine, dimethylamine, trimethylamine and butylamine and/or water.
11. The method of claim 5, wherein said supercritical fluid additionally comprises an alkylation catalyst.
12. The method of claim 1, wherein said analyte is selected from the group consisting of a drug of abuse, a pesticide, an herbicide, an environmental toxin and a carcinogen.
13. The method of claim 12, wherein said analyte is a drug of abuse selected from the group consisting of cocaine, heroin, morphine, 6-monoacetylmorphine, codeine, opium, methamphetamine, amphetamine, MDA, MDMA, PCP, Δ-9-THC, Δ-9-THC-acid, phencyclidine, and metabolites thereof.

14. The method of claim 1 or 2, wherein said subject is a human or a domestic animal.
15. The method of claim 1 or 2 comprising an additional step of washing with supercritical CO<sub>2</sub> to remove possible environmental contamination before said testing step.
16. The method of claim 1 or 2, wherein said detection step is selected from group consisting of an immunoassay test and a GC/MS test.
17. A method for a supercritical fluid extraction of an analyte having a reactive hydrogen group from a biological sample, comprising the step of treating the sample with a derivatizing agent prior to initiating the supercritical fluid extraction.
18. The method of claim 17, wherein said reactive hydrogen group is selected from the group consisting of an alcohol, an amide, an amine, an aryl and a thiol.
19. The method of claim 18, wherein said derivatizing agent is selected from the group consisting of an acylating agent, an esterification agent, an alkylating agent, a methylating agent and a silylating agent.
20. The method of claim 19, wherein said acylating reagent is selected from the group consisting of an anhydride, an acyl halide, an activated acyl amide.
21. The method of claim 20, wherein said activated acyl amide comprises acylimidazole or *bis*(acylamide).
22. The method of claim 20, wherein said acyl halide comprises a halogenated acylation reagent selected from the group consisting of a perfluoro acid anhydride, a perfluoroimidazole and an *N*-methyl-*bis*[trifluoroacetamide].

23. The method of claim 22, wherein said perfluoro acid anhydride is selected from the group consisting of trifluoroacetic acid anhydride (TFAA), pentafluoropropionic acid anhydride (PFPA) and heptafluorobutyric acid anhydride (HFBA).
24. The method of claim 22, wherein said perfluoroimidazole is selected from the group consisting of trifluoroacetylimidazole (TFAI), pentafluoropropionylimidazole (PFPI) and heptafluorobutyrylimidazole (HFBI).
25. The method of claim 22, wherein said *N*-methyl-*bis*[trifluoroacetamide] comprises *N*-methyl-*bis*-(trifluoroacetamide) (MBTFA).
26. The method of claim 17, wherein said analyte is a drug analyte selected from the group consisting of methamphetamine, amphetamine, methylenedioxymphetamine (MDA), methylenedioxymethamphetamine (MDMA), and methylenedioxymethamphetamine (MDEA).
27. A method for a supercritical fluid extraction of an analyte having a reactive carboxyl group from a biological sample, comprising the step of treating the sample with a derivatizing agent prior to initiating the supercritical fluid extraction.
28. The method of claim 27, wherein said derivatizing agent comprises an esterification reagent.
29. The method of claim 28, wherein said esterification reagent is selected from the group consisting of a pentafluoropropanol and an anhydride.
30. A method for a supercritical fluid simultaneous extraction of two or more analytes from biological sample, wherein said analytes comprise both a reactive hydrogen group and a reactive carboxyl group, comprising the step of treating the sample with a derivatizing agent effective to derivatize both the reactive hydrogen and the reactive carboxyl.

31. The method of claim 30, wherein said derivatization agent comprises a perfluoro acid anhydride.
32. The method of claim 31, wherein said reactive hydrogen is acetylated and said reactive carboxyl is esterified.
33. The method of claim 32, wherein said esterification of the carboxylic acid groups with the acid anhydride result in the formation of a pentafluoropropyl-ester-drug analyte-derivative.
34. The method of claim 30, wherein said analyte is a drug analyte selected from the group consisting of methamphetamine, amphetamine, MDMA, MDA, cocaine, benzyoylecgonine, cocaethylene, heroin, morphine, codeine, 6-MAM, delta-9-THC, delta-9-THC-9-carboxylic acid, dihydroxyl THC, PCP, Nicotine and cotinine.
35. A method for a supercritical fluid simultaneous extraction of two or more analytes from a biological sample, wherein said analytes comprise both a reactive hydrogen group and a reactive carboxyl group, comprising the step of treating the sample with two or more derivatizing agents effective to derivatize both the reactive hydrogen and the reactive carboxyl.
36. The method of claim 35, wherein said two or more derivatizing agents are added simultaneous or sequentially to an supercritical extraction fluid.
37. The method of claim 36, comprising addition of a perfluoro-anhydride and a perfluoro-alcohol to the supercritical extraction fluid.
38. The method of claim 17, comprising the additional step of adding a modifier to said supercritical fluid.

39. The method of claim 38, wherein said modifier comprises an organic solvent selected from the group consisting of dichloromethane, dichloroethane, methanol, acetone, ethanol, ethyl acetate and methyl acetate.

40. The method of claim 17, comprising the additional step of adding an additive to said supercritical fluid.

41. The method of claim 40, wherein said additive comprises an organic amine selected from the group consisting of diethylamine, triethylamine and butylamine.

42. The method of claim 40, wherein said additive comprises an organic buffer and a polyethylene-glycol.

43. The method of claim 40, wherein said organic buffer comprises about 0.1 mM to about 10 mM Tris base.

44. The method of claim 40, wherein said polyethylene glycol comprises about 1% (v/v) to about 15% (v/v) of polyethylene glycol having a polymer molecular weight of about 1500 to about 3000.

45. The method of claim 40, wherein said additive comprises about 0.1% (v/v) to about 5% (v/v) of a zwitterionic compound.

46. The method of claim 45, wherein said zwitterionic compound is selected from the group consisting of a zwitterionic detergent, an amino acid, a glycolipid and an amino sugar.

47. The method of claim 46, wherein said zwitterionic detergent is selected from the group consisting of 3-[(3-chloramidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), 3-[(3-chloramidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), N-decyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate (NDAPS) and phosphatidyl-choline dipalmitoyl.

48. The method of claim 47, wherein said zwitterionic detergent additive further comprises about 1% (v/v) to about 20% (v/v) of an antifoaming agent.

49. The method of claim 48, wherein said antifoaming agent is selected from the group consisting of a polyol, a mixture of a silicone and a polyols and a silicone.

50. The method of claim 49, wherein said polyol comprises Antifoam 204.

51. The method of claim 49, wherein said mixture of silicones and polyols comprises Antifoam 289.

52. The method of claim 49, wherein said silicone comprises Antifoam A or Antifoam B.

53. The method of claim 38, wherein said additive comprises an anionic detergent, a cationic detergent and antifoaming agent.

54. The method of claim 53, wherein said anionic detergent is selected from the group consisting of an alginic acid, a caprylic acid, a cholic acid, a decane sulfonic acid, a dehydrocholic acid, a deoxycholic acid, a dioctyl sulfosuccinate, a dodecanesulfonic acid, a glycocholic acid, a glycodeoxycholic acid, a heptane sulfonic acid, a hexane sulfonic acid, a lauroylsarcosine, a lauryl sulfate, a nonanesulfonic acid, an octanesulfonic acid, a pentanesulfonic acid, a taurocholic acid, a taurodeoxycholic acid, a Teepol HB7, a Tergiton and a Triton.

55. The method of claim 54, wherein said alginic acid comprises a  $\beta_{1,4}$ -D-mannuronic acid polymer.

56. The method of claim 53, wherein said cationic detergent is selected from the group consisting of an alkyltrimethylammonium bromide, a benzalkonium

chloride, a benzyldimethyldodecyl ammonium bromide, a benzyldimethylhexadecyl ammonium chloride, a benzyldimethyltetradecyl ammonium chloride, a cetyltrimethylammonium bromide, a cetylpyridinium bromide, a cetylpyridinium chloride, a decamethonium bromide, a dimethyldioctadecyl ammonium bromide, a methylbenzethonium chloride, a methyl mixed trialkyl ammonium chloride, a methyl trioctylammonium chloride, and an N,N',N'-polyoxyethylene(10)-N-tallow-1,3-diamino propane.

57. The method of claim 53, wherein said antifoaming agent is selected from the group consisting of a polyol, a mixture of a silicone and a polyol and a silicone.

58. The method of claim 57, wherein said polyol comprises Antifoam 204.

59. The method of claim 57, wherein said mixture of a silicone and a polyol comprises Antifoam 289.

60. The method of claim 57, wherein said silicone comprises Antifoam A or Antifoam B.

61. A method for testing a biological sample to distinguish between environmental exposure to an analyte and metabolic incorporation of the analyte, comprising the steps of:

(i) placing said sample and an equipment packet comprising a modifier and an optional additive into an extraction chamber suitable for maintenance of both a static mode and a dynamic flow mode of a supercritical fluid;

(ii) initiating a dynamic flow mode effective to washing said sample in said extract chamber with a supercritical fluid under physical conditions of temperature and pressure that are effective to remove analyte that is environmentally associated but not analyte that is metabolically incorporated into said sample and wherein said conditions are not effective to release the modifier or additive from the equipment packet;

(iii) collecting a wash sample for an off-line testing procedure and terminating said flow of supercritical fluid to switching from said dynamic mode to a static mode;

(iv) in a static mode, changing said physical conditions to conditions of temperature and pressure effective to release said modifier and optional additive from said equipment packet and maintaining said static mode for a period of time effective to extract the metabolically incorporated analyte;

(v) switching to a dynamic mode by resuming flow of said supercritical fluid; and changing said physical conditions to conditions of temperature and pressure effective to extract the metabolically incorporated analyte;

(vi) collecting an extract sample for an off-line testing procedure;

(vii) testing said wash sample to determine the presence or amount of said environmentally associated analyte;

(viii) testing said extract sample to determine the presence or amount of said metabolically incorporated analyte;

wherein, a positive test result in step (vii) and a negative test result in step (viii) is effective to establish said environmental exposure to said analyte without metabolic incorporation; a negative test result in step (vii) and a positive test result in step (viii) is effective to establish metabolic incorporation of said analyte without environmental exposure; and, a positive test result in both step (vii) and step (viii) is effective to distinguish both environmental exposure and metabolic incorporation of said analyte into said sample.

62. A method for extracting an analyte from a biological sample comprising the step of incubating the sample with an enzyme capable of degrading a matrix glycoconjugate of the sample for a period of time and under conditions effective to hydrolyze said glycoconjugate.

63. The method of claim 62, wherein said enzyme is selected from the group consisting of a hexosaminidase, an endoglycosidase, a sialidase, a galactoaminidase, a glucosaminidase, a glucosidase, a galactosidase, a phytase and a chitinase.

64. A supercritical fluid extraction equipment packet comprising a solution selected from the group consisting of a modifier stock solution, an additive stock solution and a derivatizing agent solution.

65. The equipment packet of claim 64, wherein said modifier stock solution comprises an organic solvent selected from the group consisting of dichloromethane, dichloroethane, methanol, acetone, ethanol and ethyl acetate.

66. The equipment packet of claim 65, wherein said additive stock solution comprises, comprising one or more detergents selected from the group consisting of a zwitterionic detergent, an anionic detergent and a cationic detergent.

67. A supercritical fluid extraction method for screening a subject to determine the possible exposure to, or ingestion of, or inhalation of an animal brain product, comprising the steps of collecting a biological sample from the subject, extracting complex carbohydrates from the sample and testing to determine the presence or amount of an animal brain complex carbohydrates present in said sample.

68. The method of claim 67, wherein said complex carbohydrates are selected from the group comprising glycoprotein and glycolipid blood group antigens.

69. The method of claim 67, wherein said complex carbohydrate comprises a glycolipid selected from the group consisting of a neutral glycolipid, a ganglioside and a sulfatide.

70. A method for simultaneously extracting an analyte from a biological sample having glycoconjugate matrix comprising the steps of treating the sample with a derivatizing agent effective to neutralize the chemical charge of one or more sugar ring substituent groups in said glycoconjugate matrix; and, extracting the analytes in a supercritical fluid.

71. A supercritical fluid extraction method for extracting an analyte from a biological sample, comprising the step of bringing the sample into contact with a modifier solution comprising an anionic detergent, a cationic detergent and an organic solvent, for a period of time and under conditions effective to extract said analyte from said sample.

72. The method of claim 1, 2, 17, 27, 30, 35, 61, 62, 67, 70 or 71, wherein the biological sample is selected from the group consisting of hair, feathers, nails, hoofs, skin and muscle.

73. The method of claim 1, 2, 17, 27, 30, 35, 61, 62, 67, 70 or 71, wherein the analyte is selected from the group consisting of a polar, a non-polar analyte and a combination of both.

74. The method of claim 1, further comprising exposing the sample to a derivatizing agent in an amount effective to form derivatives of the analyte.

75. The method of claim 74, wherein the analyte is a polar analyte.

76. The method of claims 1, 2, 17, 27, 30, 35, 61, 62, 67, 70 or 71, wherein the analyte is selected from the group consisting of a drug analyte and a chemical analyte.